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Insecticide Resistance Management and Integrated Mite Management in Orchards: Can They Coexist?*

Larry A. Hull,† Bruce A. McPheron & Amy M. Lake

Penn State University, Department of Entomology, Fruit Research and Extension Center, PO Box 309, Biglerville, PA 17307-0309, USA

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Abstract: At the core of an integrated pest management program for Pennsylvanian apple orchards is an integrated mite management program that is based on a natural enemy, the coccinellid *Stethorus punctum punctum* (LeConte). The program relies upon the principles of ecological selectivity (e.g. chemical selection, timing, dose and method of application) for the organophosphate and carbamate insecticides. During the last 20 years the tufted apple bud moth (TABM), *Platynota idaeusalis* (Walker), a direct pest of apple, has developed resistance to these two chemical classes. In an effort to address this growing resistance problem, an intensive research program was initiated in 1986 on how to manage insecticide resistance in TABM while preserving the integrity of the integrated mite management program. One aspect of this research program is the investigation of biochemical and genetic approaches to resistance, including an analysis of detoxification mechanisms, effects of host plant allelochemistry on resistance and detoxification enzyme activities, reversion, gene flow and the isolation and characterization of a gene for glutathione transferase from TABM. Management approaches that have been developed and successfully implemented include pheromone mating disruption, parasitoids, ground-cover management including insecticides, *Bacillus thuringiensis* Berl. products and insect growth regulators. Resistance management options for TABM are presented.

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† To whom correspondence should be addressed.

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1 INTRODUCTION

An integrated mite management (IMM) system for apples in Pennsylvania, USA was started in 1968 due to the difficulty in controlling two species of phytophagous mites, the European red mite, *Panonychus ulmi* (Koch) and the two-spotted spider mite, *Tetranychus urticae* Koch.[1] Both species had developed resistance to many of the acaricides available at that time. The IMM system was based upon the biological control of these two species by the native coccinellid, *Stethorus punctum punctum* (LeConte), with acaricides used only to manipulate the predator : prey ratio. The cornerstone of this system was the use of selectively timed applications of low rates of organophosphate insecticides and fungicides, which were applied using the alternate row middle application method to control the major insect and disease pests.[2] This management system resulted in orchard pesticide reductions of approximately 80% in acaricides, 50% in insecticides and 40% in fungicides.[3]

The tufted apple bud moth (TABM), *Platynota idaeusalis* (Walker), is a tortricid pest of apples, cherries, nectarines and peaches in Pennsylvania. It is bivoltine with second-brood larvae usually responsible for the major fruit injury seen on apples at harvest.[4] The larvae overwinter as second through fifth instars in shelters located in the ground cover beneath fruit trees and in habitats outside the orchard. They complete their development in the spring on a wide variety of herbaceous species and apple root suckers before the adults begin flying into the trees to mate and oviposit.[5]

In the late 1970s growers began to experience increasing amounts of fruit injury from TABM. In the major growing regions of Pennsylvania, fruit injury from TABM steadily increased from 0·3% in 1973,[6] to 3·0% in 1979,[3] to 6·1% in 1986.[5] More recent estimates include damage levels from 8·0 to 13·0% (Hull, L. A., unpublished). A number of reasons were cited for this increased fruit injury, including poor timing of insecticide applications, early-season cessation of spray programs, improper pruning of trees and lack of a lethal residue on the offside of trees sprayed by the alternate row middle method.[7] In addition, our recent studies suggest that in Pennysylvania and a number of other states in the eastern U.S., TABM has developed up to a 20-fold level of resistance to the organophosphate insecticide azinphos-methyl.[5,8,9] Further data suggest that increased resistance to other registered organophosphate insecticides (microencapsulated parathion-methyl—42-fold, phosmet—5·6-fold and chlorpyrifos—11·2-fold) is present in this region (Hull, L. A., unpublished and Reference 10). These increased resistance levels have resulted in field failures of organophosphate insecticides in some areas.[11]

In an effort to mitigate fruit injury from TABM in the early 1980s, growers were encouraged to use insecticide combinations consisting of an organophosphate and the carbamate, methomyl.[12] Because methomyl is quite

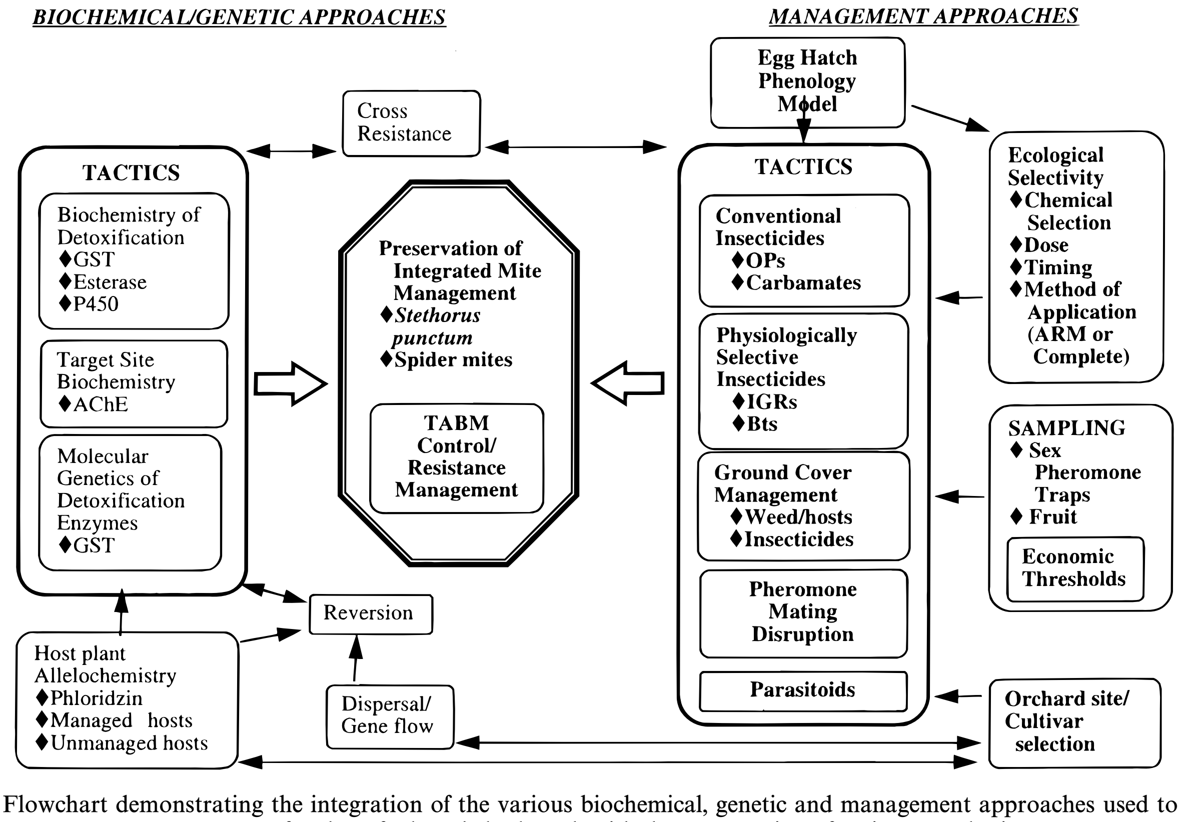

Fig. 1. Flowchart demonstrating the integration of the various biochemical, genetic and management approaches used to develop a resistance management program for the tufted apple bud moth with the preservation of an integrated mite management program.

toxic to *S. p. punctum* and the predatory mites *Amblyseius fallacis* (Garman) and *Zetzellia mali* (Ewing), and in order to preserve the integrity of the already successful IMM program, methomyl was recommended only at selected times and at 25–40% of the label rate. Unfortunately, growers began experiencing some control failures with this combination approach in the 1990s (Hull, L. A., unpublished). Hull *et al.*[13] have recently documented resistance to methomyl with levels near 17-fold in Pennsylvania.

In 1986, we initiated an extensive research program to examine a number of biochemical, genetic and management approaches to combat insecticide resistance in TABM while preserving the IMM program in Pennsylvania (Fig. 1). It is our intention from this continuing research effort to develop a management program for TABM that allows fruit growers to re-establish successful TABM control, without further exacerbating the organophospate and carbamate insecticide resistance situation. We hope that this research will lead to a proactive approach to the management of resistance to new chemistries important for TABM control.

2 BIOCHEMICAL AND GENETIC APPROACHES

2.1 Biochemical mechanisms

An investigation of the biochemical mechanisms of azinphos-methyl resistance in TABM revealed a role for increased detoxification, but no evidence of an altered target site.[14] That study found elevated glutathione transferase (GST) activity against one model substrate (dichloronitrobenzene) in adult male moths sampled from resistant orchard populations compared to sympatric susceptible populations in unmanaged habitats. We had previously identified a difference in general esterase activity between an azinphos-methyl-resistant and a susceptible laboratory population,[15] but esterase levels were inconsistent in relation to resistance and susceptibility in field populations.[14] However, Bush *et al.*[16] and Karoly *et al.*[17] found increased esterase activities in resistant populations from North Carolina and Biddinger *et al.*[9] used the esterase synergist *S, S, S*-tributylphosphorotrithioate to increase susceptibility in a resistant population from Pennsylvania, so we suggest a role of both GST and esterase in response to this pesticide.

2.2 Plant chemistry and insecticide resistance

Pesticide resistance evolves in the context of the environment, and an important part of the environment for any phytophagous insect is the array of plant allelochemicals in the host or hosts. TABM is highly polyphagous, and individual larvae may face a variety of chemicals during their lifetime. Certainly, progeny from a single female are likely to feed on chemically distinct diets due to larval dispersal.[18] Evaluation of the effects of four TABM hosts on survival and development, detoxification enzyme activity and insecticide resistance demonstrated a complex response.[19] Development rates differed among larvae fed on the four hosts, and both dandelion and black raspberry produced male-biased sex ratios. GST and esterase levels varied with hosts, as did levels of resistance to azinphos-methyl. For example, susceptible larvae that fed on dandelion were resistant to azinphos-methyl by diet-incorporation bioassay, while resistant larvae that fed on apple trees were similar to the susceptible strain in their response to azinphos-methyl.[19] However, no clear patterns of covariation between enzyme activity and mortality were noted for larvae fed on different hosts.

Hunter *et al.*[20] examined the direct role of the dihydrochalcone glycoside, phloridzin, one of the major apple allelochemicals, on TABM detoxification enzyme and azinphos-methyl resistance levels. There was a significant inhibition of larval GST activity associated with the presence of phloridzin in the diet. Esterase activity increased significantly when resistant larvae fed on phloridzin, but there was no change in two measures of cytochrome P-450 activity in this strain. Mortality of third-instar larvae was not affected by the presence of phloridzin in the diet, but neonates were more tolerant of high azinphos-methyl concentrations in the presence of phloridzin (although there were no phloridzin-dependent differences in the neonate response to lower concentrations). These results suggest that both GST and esterases may be involved in TABM response to azinphos-methyl. Because the resistance mechanism probably evolved in apple, and, thus, in the presence of phloridzin, it might also be true that one particular GST isozyme with a higher affinity for azinphos-methyl and reduced inhibition by phloridzin could be responsible for field resistance.

2.3 Molecular genetics of detoxification

This hypothesis has led us to isolate and characterize GST genes from TABM. A full-length transcript for a class 2 GST has been isolated from a TABM cDNA library using a *Manduca sexta* (Joh.) GST probe[21] by E. J. Carlini as part of his dissertation research. He has expressed the protein in an *Escherichia coli* Cast. & Chalm. expression system and will characterize the activity of this isozyme toward azinphos-methyl (Carlini, E. J. pers. comm.). GSTs are multigene families, so it is quite possible that this particular gene is not responsible for azinphos-methyl resistance in this insect. However, we can still learn much about how expression of this GST gene is affected by plant allelochemicals.

Also, possession of a clone from TABM will facilitate the search for other members of this GST class.

2.4 Gene flow

Two topics that are central to most discussions of resistance management are gene flow and reversion to susceptibility.[22] Gene flow affects the distribution of resistance alleles throughout different populations of an organism,[23] and it is also important for the movement of susceptible genotypes from refugia back into managed habitats. Knight & Hull[5] and Knight *et al.*[24] examined male and female TABM dispersal *via* mark-release-recapture studies. They showed an asymmetry of dispersal distance between males and females, but the species displays genetically significant dispersal capability. Dispersal by first-instar TABM larvae is also potentially important because larvae are capable of moving between habitats as a function of wind velocity and direction.[18] An allozyme study of TABM populations (McPheron, B. A., unpublished) showed no significant population substructure across a range of habitats in an area of south-central Pennsylvania. This result is consistent with the dispersal capabilities of the insect—there do not appear to be barriers to gene flow. The heterogeneity in insecticide resistance levels among local habitats[25] is probably a consequence of local variation in selection pressure from pesticides and effects on resistance levels dictated by interactions with host-plant chemistry.

2.5 Reversion

Reversion of a resistant TABM line toward susceptibility has been observed in the laboratory (Knight, A. L. & Hull, L. A., unpublished and Reference 16). To examine reversion in the field, we have conducted a study of resistance levels and fitness parameters of an azinphos-methyl-resistant population on caged apple trees over six generations of differential exposure to the pesticide. Although interpretation of results from the final year was complicated by a viral epizootic, we observed no change in resistance across treatments varying from zero dose to high levels of pesticide (Lake, A. M., unpublished). The data suggest that reversion in the laboratory (Knight, A. L. & Hull, L. A., unpublished) could be due to selection for a different mechanism of resistance from that found in the field, at least for populations in south-central Pennsylvania, and that dispersal of susceptible genotypes from refugia may be an important part of the dynamics of area-wide resistance patterns in this insect.

One reason to examine the biochemical and genetic bases of resistance, even in a case where that resistance is so widespread that continued use of the compound is problematic, is to help us design strategies that minimize the likelihood of resistance to new management tactics. We are particularly concerned about the potential of cross-resistance to recently discovered, as yet non-registered compounds (e.g. insect growth regulators). Biddinger *et al.*[9] showed that azinphos-methyl-resistant TABM were also resistant to fenoxycarb, but not to a range of other insect growth regulators or to abamectin. Recent studies[13] have shown that methomyl resistance is now widespread among TABM populations in south-central Pennsylvania. We are still investigating the potential cross-resistance of this situation. The existence of pervasive, stable resistance to past management practices is a constant reminder of the need to identify novel management tactics that provide a variety of selection pressures and to consider carefully the deployment of these tactics to maximize their efficacy and life span.

3 MANAGEMENT APPROACHES

3.1 Phenological egg hatch model

To time more precisely insecticide applications for neonate larvae, the most susceptible stage of TABM,[26] and minimize selection pressure, we initiated a field study to construct a phenology model to predict accurately the egg hatch periods for both broods of TABM. A 14-year-old apple orchard in Arendtsville, PA, consisting of 'Red Chief Delicious' and 'Commander Yorking' cultivars, served as the site for the study (1992–1995). Degree days (base 7·2°C min., 32·8°C max.) were calculated on-site using daily maximum and minimum temperatures taken from a Climatronics® meteorological instrument located at a weather station next to the orchard. From mid–late May through early September of each year, degree days and hatch dates for the more than 100 egg masses found and tagged within the orchard during timed searches were recorded. A simple linear model describing the relationship between degree day accumulation from a biofix point (first sustained capture of adult males in a sex pheromone trap) and hatch dates was created for each year of the study. A model, combining 1992 and 1993 data, was developed to predict egg hatch dates for 1994 and 1995. Data from 1994 and 1995, in turn, served to validate the model described in 1992/1993. This model is currently being used to make spray-timing recommendations for fruit growers.[27]

3.2 Ground cover management

Field studies have shown that the spatial distributions in the orchard ground cover of the mite predator, *S. p. punctum* and of TABM exhibit seasonal variation.[28,29] In the fall, the distribution of these two species is similar; both are found primarily in the herbicide-treated strip around the base of the trees associated with fallen leaves and root suckers, although many of

the coccinellid adults congregate near the apple tree trunk. In the spring, the spatial distributions of *S. p. punctum* adults and TABM larvae are not similar; $>70\%$ of the beetle adults are found in the trunk zone associated with fallen leaves and root suckers,[28] while the leafroller larvae are found near the sod row middles closely associated with the fallen leaves.[29] By the petal-fall stage of apple development, the movement of *S. p. punctum* adults from their overwintering sites in the ground cover into the trees is complete. This mass exodus occurs before overwintering TABM larvae begin to pupate.[30] Thus, it is possible to manage overwintering TABM larvae in the ground cover without disrupting the endemic *S. p. punctum* population if (1) fallen leaves collected around the trunks are not disturbed prior to petal fall, and/or (2) an application of esfenvalerate to the orchard ground cover is delayed until petal fall when the predator has moved into the apple tree.[29]

3.3 Pheromone mating disruption and parasitoids

Through large and small plot orchard trials conducted in Pennsylvania commercial apple orchards from 1991 to 1996 we have found that the use of pheromone mating disruption provides adequate control of low to moderate TABM populations without the use of insecticides after petal fall (Reference 31 and Hull, L. A., unpublished). This reduction in insecticide input allows predatory mites and *S. p. punctum* to maintain the phytophagous mites below economic injury levels, which, in turn, maintains the integrity of the IMM program.[31] In addition to reducing insecticide inputs, mating disruption appears to enhance the survival of a large complex of leafroller parasitoids in these orchards; Hull *et al.*[32] found parasitism levels of TABM larvae near 27% during August in mating disruption blocks, in contrast to no parasitism in conventionally treated blocks during the same period. Surveys of the leafroller parasitoid fauna in various Pennsylvania apple orchards under varying levels of pesticide management from 1990 to 1993 revealed an abundant complex of leafroller parasitoids. A total of 41 species were found to attack TABM larvae and pupae and, secondarily, other leafrollers.[33] Because mating disruption can reduce insecticide inputs, thereby reducing insecticide selection pressure while conserving high numbers of important natural enemies of both phytophagous mites and TABM, we have incorporated this important component into our IMM and TABM resistance management programs wherever low to moderate TABM populations exist.

3.4 *Bacillus thuringiensis*

With the recent introduction of new commercial products containing *Bacillus thuringiensis* Berliner (B.t.), a naturally occurring soil bacterium that produces crystalline proteins with insecticidal properties, we re-evaluated this approach for TABM management. These biorational compounds attack the insect midgut and are very selective, not only toward insects, but toward specific insect orders. This selectivity would allow us to integrate B.t. products as management tools for TABM without disrupting the IMM program based on *S. p. punctum* or the other beneficial arthropods found in orchards. From 1992 to 1995, we conducted a series of experiments containing various B.t. products to examine their efficacy for TABM control and their effects on non-target organisms.

In 1992, a B.t. suspension ('MVP', a CrylAc toxin encapsulated in killed *Pseudomonas* cells; Mycogen, San Diego, CA) provided control similar to tebufenozide, an insect growth regulator, and a combination of phosmet plus methomyl for both generations of TABM, although the two latter treatments were applied only twice and four times, respectively, in comparison to a total of six applications for MVP.[34] In 1993, the residual properties of five B.t. compounds were examined using a leaf-disc bioassay. All compounds had very little residual activity in comparison to encapsulated parathion-methyl; activity of B.t. products against TABM neonate larvae began to decline as early as three to six days after the leaves were sprayed.[35] In 1994 and 1995, several B.t. products were evaluated in a series of orchard trials to examine TABM efficacy and impact upon natural enemies. All products tested gave control of TABM equivalent to other conventional organophosphate plus methomyl combinations and had no negative impact on the natural enemy populations in the orchards.[36–38]

3.5 Insect growth regulators

Rising levels of resistance by TABM to registered broad-spectrum insecticides, such as the organophosphates and the carbamate methomyl, are causing an increase in fruit injury and are threatening the continuing success of Pennsylvania's IMM program because of an increase in the amount of insecticides required to achieve control. Unless this trend can be reversed, allowing growers to once again effectively control TABM with these chemicals, new chemistries that are effective and compatible with the IMM program will have to be found as replacements. With the assumption that reversion to a more susceptible level is rare in field populations, we started examining the efficacy of a newer class of physiologically selective chemicals, insect growth regulators (IGR), for control of TABM in the mid-1980s.

Fenoxycarb, a juvenile hormone analog that mimics the insect's naturally occurring juvenile hormone, was one of the first IGRs we examined for TABM control. When applied to ultimate instar larvae, pupation is

often delayed, which results in abnormal pupae that die before eclosion or adults that cannot feed or reproduce.[39] However, when fenoxycarb is applied to early- or mid-instar larvae, abnormally large larvae can occur through the production of supernumerary molts.[39] These larvae, in turn, consume more plant tissue over a longer period of time, thus causing more damage. Due to the extreme stage-specificity of the chemical, the timing of a fenoxycarb application is critical. We found that an application of fenoxycarb improperly timed for TABM caused an increase in fruit injury.[39] Also, Biddinger and Hull[40] reported that fenoxycarb was harmful to the eggs, larvae and pupae of *S. p. punctum*, a finding that makes this chemical incompatible with our IMM program.

Several ecdysone agonists—IGRs that mimic the natural insect molting hormone, ecdysone—have been tested for TABM control over the past ten years. RH-5489 (*N′*-benzoyl-*N*-*tert*-butylbenzohydrazide) was the first compound to be examined, but was quickly replaced with a more efficacious, closely related compound, tebufenozide (RH-5992; *N*-*tert*-butyl-*N′*-(4-ethylbenzoyl)-3,5-dimethylbenzohydrazide). Tebufenozide is extremely active against the codling moth, *Cydia pomonella* (L.) and the leafroller complex (including TABM) in Pennsylvania.[37,41] Our research indicates that two applications of tebufenozide provide a much higher level of leafroller control than four applications per season of various organophosphate/carbamate combinations or five applications of *B. thuringiensis* products.[38] The product has no deleterious effects on non-target organisms, particularly *S. p. punctum* and predatory mites, and is therefore an ideal chemical for use with Pennsylvania's IMM program.

With the understanding that insecticide resistance is inevitable and that it can only be delayed, we launched a series of laboratory and field experiments in an attempt to better understand how tebufenozide works against TABM, and how resistance to this compound might develop, before it is registered for commercial use. An on-going laboratory study attempts to select for resistance in several field-collected populations of TABM from Pennsylvania and West Virginia by continuously rearing larvae on artificial diet treated with tebufenozide. Other studies include field and laboratory tests that look at the sublethal effects of tebufenozide on TABM populations. We hope that the information gathered from these projects will allow us to be proactive, rather than reactive, in our attempts to delay resistance by TABM to tebufenozide.

4 RESISTANCE MANAGEMENT OPTIONS FOR THE TUFTED APPLE BUD MOTH

1. Plant smaller block units of apples intermixed with other deciduous fruits to preserve refugia for susceptible individuals.
2. Practice good ground-cover management within the orchard (i.e. eliminate broadleaf weeds and dropped fruit within tree drip line) and apply esfenvalerate to the orchard ground cover within the drip line at pink bud or petal fall.
3. Keep trees well pruned, remove water sprouts (water shoots) and thin fruit properly.
4. Use correct application procedures (i.e. alternate row middle or complete sprays), calibrate sprayer to match tree size and use the proper volume of water per tree to ensure coverage.
5. Spray only when economically necessary (i.e. use action thresholds based on sex pheromone traps or fruit injury)[26] and use the lowest most effective rates compatible with IPM.
6. Use an egg degree day model to properly time sprays to kill most susceptible stages (i.e. eggs and neonate larvae)—DO NOT TREAT FOR LARGE LARVAE.
7. Alternate among insecticide classes between generations where possible or use combinations of insecticide classes within generations to promote integrated mite management.
8. Chemical options:
   (a) alternate between organophosphates and methomyl between generations, *or*
   (b) combine organophosphates and methomyl within one generation only, *or*
   (c) use tebufenozide for one generation only, preceded/followed by another option, *or*
   (d) use *Bacillus thuringiensis* products for one generation only, preceded/followed by another option.
9. Use pheromone mating disruption in conjunction with, or as a replacement for, insecticides when attempting to control low to moderate populations.

5 CONCLUSIONS

We conclude by returning to the question posed in our title: can insecticide resistance management and integrated mite management co-exist in orchards? We believe that the answer is yes, to varying degrees. Without the introduction of new chemistries to control TABM, our success will be dependent on the adoption of alternative tactics (e.g. mating disruption, ground-cover management) for the control of this pest, because we must maintain commercially acceptable control of the other direct fruit pests. While this option is likely to preserve the integrity of the IMM program, it will probably not suppress or reverse the levels of resistance to the organophosphate and carbamate chemistries that exist. Our chances of success in maintaining integrated mite management and TABM management will be greatly enhanced if new, physiologically selective, chemistries for TABM control are identified and registered in the US. We, as researchers, will then have the

responsibility to develop resistance management strategies for these chemicals that can be in place by the time they are registered. Grower cooperation with this pro-active approach will help prolong the effective life of new, more ecologically compatible pest management strategies.

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